CleanCap®

Accelerated manufacturing of messenger RNA (mRNA) vaccines using CleanCap® co-transcriptional capping

TriLink BIOTECHNOLOGIES

part of **Maravai LifeSciences**

A rapidly scalable platform for vaccine development

Accelerated timelines are critical when responding to an outbreak. TriLink has pioneered a manufacturing process for mRNA vaccines—using CleanCap technology—that is cell-free, rapid, and highly scalable.

mRNAs allow biotechnology companies to develop vaccines in response to pandemics, such as COVID-19, in a matter of weeks.

Vaccines produced using CleanCap technology benefit from a streamlined, high-yield production pipeline that requires fewer manufacturing steps, all at a lower cost than traditional methods.

CleanCap technology can help you reach the clinic faster.

State of the art: How mRNA vaccines work

mRNA vaccines work similarly to live attenuated and subunit vaccines by mimicking viruses, such as SARS-CoV-2, and using the patient's own immune system to fight the perceived infection. Unlike traditional vaccines, mRNA vaccines harness the patient's cellular translation machinery to produce the antigen. There are currently two popular variants of mRNA vaccine technology: non-replicating mRNA and self-amplifying RNA (saRNA).

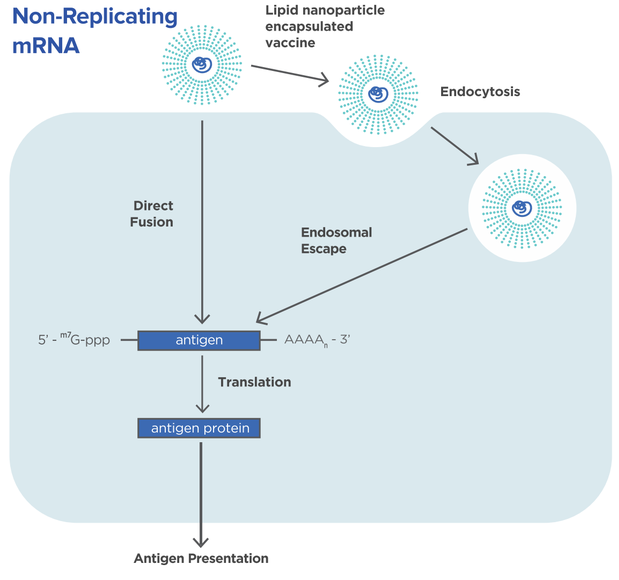
mRNA vaccines elicit a protective immune response in two steps:

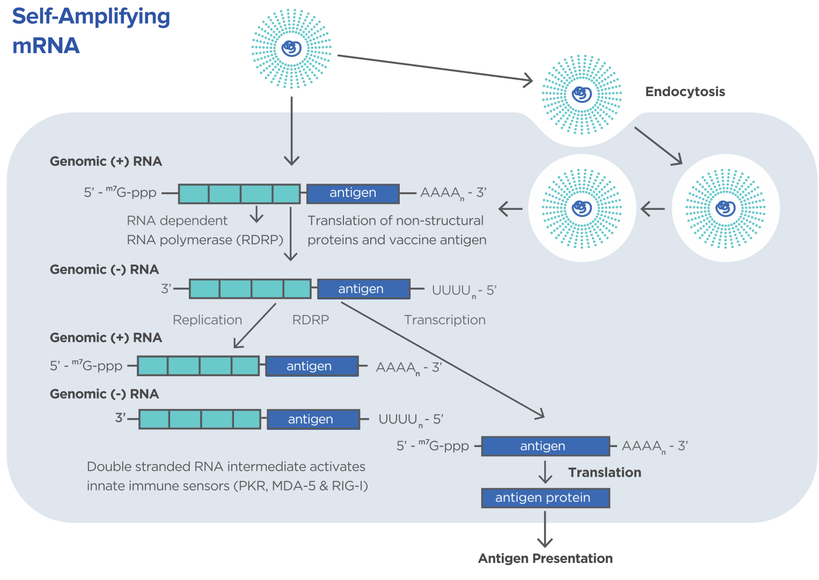
Step 1

The mRNA enters the cell via endocytosis or direct fusion of the lipid nanoparticle. After it reaches the cytoplasm within the cell, the mRNA is translated to produce a viral antigen. saRNAs include additional components that duplicate the mRNA, lowering the dose needed to generate the same antigen levels.

Step 2

The viral antigen is presented to the immune system, leading to an immune response and memory generation that protects the patient from future exposure to the virus. saRNAs boost the immune response by producing double-stranded RNA that serves as an adjuvant.





The antigens introduced by the mRNA vaccine stimulate a natural immune response that generates protective memory. Quickly immunizing the population is an important component of pandemic response efforts.

mRNA vaccines are created by in vitro transcription, which features a modular development and manufacturing process. This allows them to be rapidly deployed against novel disease targets.

The benefits of mRNA capping

mRNA vaccine effectiveness depends on the stability and successful translation of injected mRNAs. However, there are many cellular receptors that detect foreign mRNA and lead to its silencing. mRNA cap structures, or structured 5’ ends, are a key feature of native mRNAs that must be replicated in order to evade immune detection and ensure antigen expression.

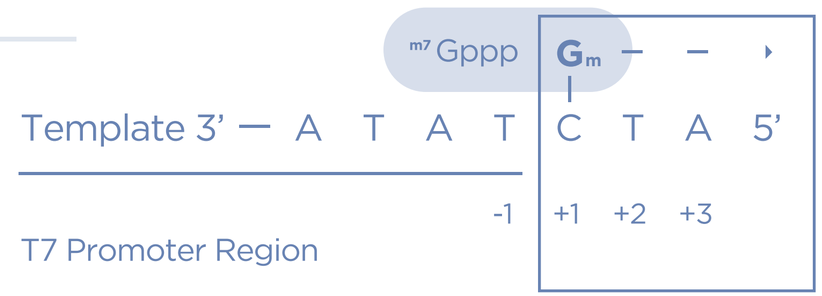
Natural capping proceeds in eukaryotic cells through a series of enzymatic steps that occur during transcription. The 5’ guanosine (G) is methylated first, resulting in a Cap 0 structure. A second methyl group is then added to the +1 ribonucleotide to generate the Cap 1 structure.

The Cap 0 structure provides stability and translational benefits to mRNAs, but does not protect against immune-mediated silencing. The Cap 1 structure cloaks mRNA from immune detection by cellular RNA sensors and subsequent silencing. This makes the Cap 1 structure the optimal choice when manufacturing mRNA vaccines.

Uncapped

Cap 0

Cap 1



Current mRNA capping strategies are either costly at scale, inefficient, or unable to produce the beneficial Cap 1 structure. CleanCap technology combines cost savings at scale with accelerated timelines and high capping performance.

Based on an alphavirus backbone, saRNA requires a different capping strategy than non-replicating mRNA. CleanCap AU is a cap analog tailored for saRNA that retains the authentic alphavirus 5′ ends.

Traditional capping methods in the marketplace

Messenger RNA capping is conventionally accomplished using two approaches:

01 ARCA capping

Anti-Reverse Cap Analog (ARCA) is a cap analog that generates a Cap 0 structure. It is added to the mRNA transcription reaction, resulting in a co-transcriptional capping process.

ARCA uses guanosine (G) as an initiating nucleoside for in vitro transcription by T7 RNA polymerase, which can be nonideal. ARCA capping results in a low capping efficiency rate (~70%) and a subpar transcription yield (~1.5 mg/ml).

02 Enzymatic capping

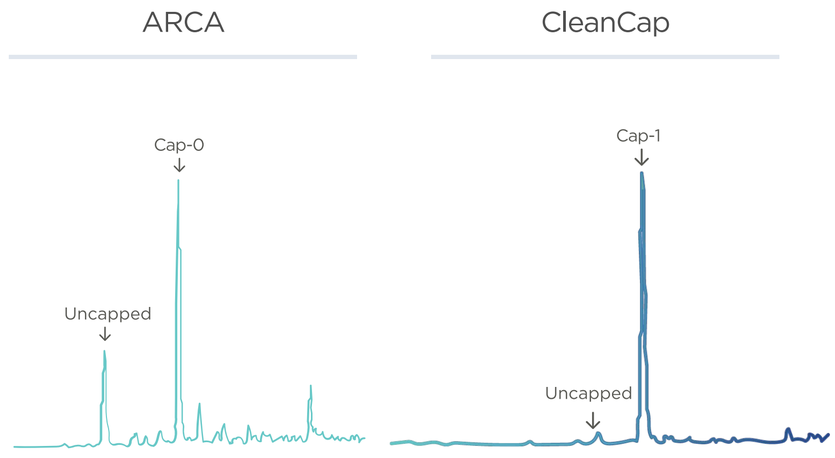
This method uses a set of enzymes from the Vaccinia virus to cap mRNA in a separate reaction after transcription has occurred. This adds more manufacturing steps, especially if researchers purify the initial uncapped transcript prior to enzymatic capping.

These enzymes are expensive to scale and can vary in their capping efficiency. Enzymatic capping also includes heating and extra purification steps that can degrade mRNAs.

The CleanCap solution to mRNA vaccine capping

Vaccine development scientists no longer need to choose between mRNA performance and affordable manufacturing. CleanCap technology yields a natural Cap 1 structure via co-transcriptional capping, eliminating the prohibitive costs of legacy enzymatic methods. This next-generation technology offers an extremely high capping rate of >95% and a high crude yield of ~4 mg/ml. By comparison, ARCA produces a Cap 0 structure with a capping rate of only 70% and nearly threefold less yield.

CleanCap is the ideal capping reagent for mRNA vaccines. Not only is the manufacturing process highly efficient — and capable of being deployed rapidly to combat emerging viral threats — the Cap 1 structure offers superior in vivo performance. The high capping rate and in vivo activity associated with CleanCap can reduce the effective therapeutic dose of your mRNA vaccine.



LC-MS capping analysis showing the higher capping level and desirable Cap 1 structure achieved by CleanCap vs. ARCA

CleanCap

1 Pot Transcription → Purification → Final Processing

Enzymatic Capping

Transcription → Diafiltration → Capping → Purification → Final Processing

Compared to enzymatic capping, CleanCap's one-pot synthesis reduces overall production time, saves money, and improves yield by eliminating two costly manufacturing steps.

CleanCap analogs for any mRNA sequence

TriLink has developed multiple CleanCap analogs with different initiating nucleosides for in vitro transcription. This flexibility enables researchers to select the optimal capping reagent for their mRNA sequence. AG, GG, and AU initiation sequences are all possible with CleanCap technology.

CleanCap AG

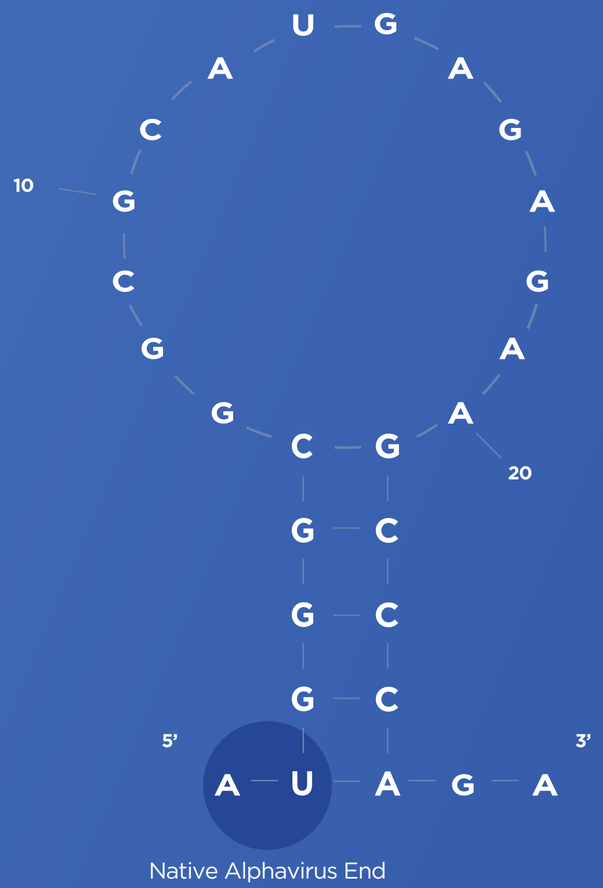
CleanCap AG offers high capping efficiency and yield with optimal co-transcriptional synthesis conditions.

CleanCap GG

CleanCap GG enables ARCA users to easily switch to CleanCap without modifying their sequence.

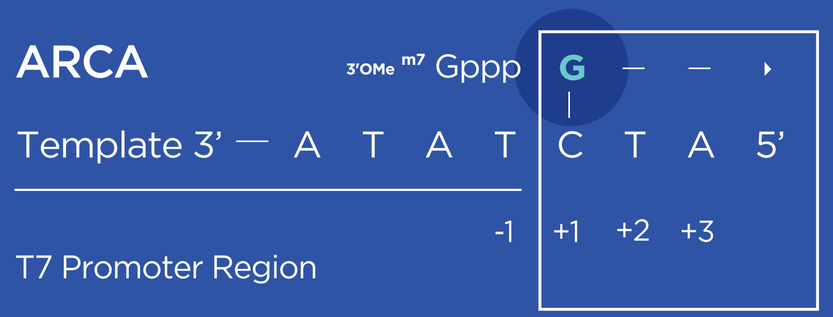
CleanCap AU

CleanCap AU is an optimized capping reagent for self-amplifying RNA that preserves the authentic alphavirus 5’ end. Learn more on the next page.



The CleanCap solution for self-amplifying RNA capping

The 5’-AU dinucleotide of saRNAs is highly conserved. Traditional co-transcriptional capping methods like ARCA produce a 5’-G end, disrupting this important sequence.



Capping with a trimer like CleanCap allows for the use of bases other than guanosine at the 5’ end, maintaining the native 5’ alphavirus structure for self-replication.

CleanCap technology enables rapid mRNA vaccine development at scale

The rapid emergence of viral pandemic threats calls for a streamlined vaccine development and manufacturing process. Efficient vaccine manufacturing enables a quicker transition from research to the clinic, saving lives.

TriLink is the leading contract manufacturer of research through GMP-grade mRNAs.

Our CleanCap technology streamlines messenger RNA vaccine production by:

- Reducing the number of manufacturing steps
- Reducing overall manufacturing cost
- Improving capped mRNA yields
- Accelerating vaccine development

CleanCap is your one-stop solution.

To learn more about TriLink, mRNAs and CleanCap technology, visit our website: trilinkbiotech.com/cleancap